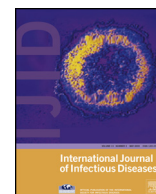


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Pneumococcal acute otitis media in infants and children in central Romania, 2009–2011: microbiological characteristics and potential coverage by pneumococcal conjugate vaccines



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SUMMARY

Objective: To assess the epidemiological and microbiological characteristics of pneumococcal acute otitis media (AOM) in children in Brasov, Central Romania, before the introduction of pneumococcal conjugate vaccine (PCV) into the routine national immunization program.

Methods: All AOM patients aged <5 years who underwent tympanocentesis or presented with purulent otorrhea of ≤24 h duration during 2009–2011 were enrolled.

Results: Two hundred and twelve consecutive AOM patients had a middle ear fluid (MEF) culture performed; 99 (46.6%) episodes occurred in patients <12 months of age. One hundred and eleven (52.4%) episodes were culture-positive. Tympanocentesis was performed in 142 patients and spontaneous otorrhea cultures in 70 patients. Overall, 114 pathogens were recovered: *Streptococcus pneumoniae* was the most common isolate (81 isolates, 70.3% of all culture-positive episodes), followed by non-typeable *Haemophilus influenzae* (26, 20.7%), *Streptococcus pyogenes* (5, 4.5%), and *Moraxella catarrhalis* (2, 1.8%). Antibiotic susceptibility and serotyping were performed for 48 (59.3%) *S. pneumoniae* isolates: 45 (93.8%) were non-susceptible to penicillin (minimal inhibitory concentration (MIC) ≥2.0 µg/ml in 24, 53.3%) and 37 (77.1%) isolates had ceftriaxone MIC values ≥0.5 µg/ml (16 with MIC >2.0 µg/ml). *S. pneumoniae* non-susceptibility rates to trimethoprim-sulfamethoxazole, erythromycin, and clindamycin were 75.0%, 58.3%, and 35.4%, respectively. All isolates were susceptible to chloramphenicol. Multidrug resistance was found in 33 (68.7%) isolates. The most common *S. pneumoniae* serotypes were 19F (14, 29.2%), 6B (8, 16.7%), 23F (8, 16.7%), and 14 (6, 12.5%). Serotype 19A was found in three (6.2%) patients and 6A in two (4.1%). Non-PCV13 serotypes represented six (12.6%) of all serotypes (four of them non-susceptible to penicillin). Thirty-six (75.0%) isolates were potentially covered by PCV7, 37 (77.0%) by PCV10, and 42 (87.5%) by PCV13.

Conclusions: (1) *S. pneumoniae* was the most prevalent pathogen, with frequent antibiotic resistance and multi-resistance patterns; (2) most pneumococcal AOM and multidrug-resistant episodes could be prevented by PCVs.

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1. Introduction

Acute otitis media (AOM) is the most frequent bacterial disease of childhood, affecting millions of children worldwide and remaining a major public health problem.^{1–3} The most common causative agents in AOM are *Streptococcus pneumoniae*, non-typeable *Haemophilus influenzae* (NTHi), *Moraxella catarrhalis*, and *Streptococcus pyogenes*.^{4–8} Together, *S. pneumoniae* and NTHi account for 60–80% of the AOM pathogens.⁹ Antibiotic resistance

is high in pneumococcal AOM, with penicillin- and amoxicillin-non-susceptible strains accounting for 30–70% of cases.^{10–16} The most commonly encountered *S. pneumoniae* serotypes in AOM are 6A, 6B, 14, 19A, 19F, and 23F.^{15,16} The highest antibiotic non-susceptibility is found in vaccine serotypes 6A, 6B, 9V, 14, 19A, 19F, and 23F.^{11,12,15,16}

Information on antibiotic resistance patterns and serotype distribution of *S. pneumoniae* isolates in infants and young children in Romania is limited. In a multinational study, 42% of *S. pneumoniae* AOM isolates from Romania were intermediately or fully resistant to penicillin.¹⁷ In three studies investigating pneumococcal mucosal and invasive disease isolates in HIV-negative and HIV-positive infants and children in northeastern

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Romania, high rates of recovery of serotypes 19A and 23F and of multidrug-resistant (MDR) organisms were reported.^{18–20} Recently, high rates of colonization with *S. pneumoniae* (reaching 71% in children 13–24 months of age) accompanied by high resistance rates to most of the commonly used antibiotic drugs were reported among healthy and sick infants and children <5 years old in central Romania.²¹

Active and continuous surveillance of the microbiology and antibiotic susceptibility patterns of AOM pathogens in Romania is important, particularly during the period preceding the introduction of pneumococcal conjugate vaccine (PCV). The aims of the present study were to assess: (1) the overall distribution of otopathogens and their antibiotic resistance patterns; and (2) PCV coverage of *S. pneumoniae* middle ear fluid (MEF) isolates in Brasov, central Romania, during 2009–2011.

2. Patients and methods

We conducted a prospective epidemiological study between January 1, 2009 and December 31, 2011, at the Children's Hospital of Brasov, in the central part of Romania. Brasov has a population of 400 000 inhabitants and the hospital is the only medical center providing medical care for infants and children in the city. The study protocol was approved by the institutional review board of the University of Transilvania, Brasov. Informed consent was obtained from the legal guardians of all children.

2.1. Patients and procedures

The study patients were infants and children aged <5 years diagnosed with AOM by a pediatrician, family physician, or otolaryngologist. The diagnosis was done when the patients presented with: (1) symptoms and physical findings consistent with AOM (fever, irritability, tugging of the ear, and redness and bulging of the tympanic membrane with blurring of its anatomic landmarks); (2) acute illness lasting ≤ 7 days. Bulging of the tympanic membrane (in addition to clinical symptoms of AOM) was present in all cases where a tympanocentesis was performed.

Culture specimens were obtained by either tympanocentesis or collection of pus from draining the ears (if lasting ≤ 24 h before enrollment). Patients with tympanostomy tubes were excluded from the study. In all episodes, we collected information on the patient's age, sex, the type of specimen (tympanocentesis or draining pus), and the patient's AOM history and recent antibiotic treatment. Data were obtained from the medical records of the hospital, clinical medical chart, or parent interview. None of the patients had been immunized with a PCV before enrollment. Tympanocentesis was performed by the study otolaryngologist (AM) as previously described.^{7,12}

2.2. Bacteriology

Swabs of MEF aspirates were placed in MW173 Amies transport medium (Transwab; Medical Wire and Equipment), plated immediately on trypticase agar containing 5% sheep blood and 5 μ g/ml gentamicin, and on chocolate agar, and incubated at 35 °C for 48 h in a 5% enriched CO₂ atmosphere. Identification, serotyping, and testing of antimicrobial susceptibility to penicillin and ceftriaxone (by E-test, PDM Epsilon meter, AB Biodisk, Solna, Sweden) and erythromycin, clindamycin, chloramphenicol, and trimethoprim-sulfamethoxazole (TMP-SMX) (by disk diffusion) were performed as described elsewhere, in accordance with the Clinical and Laboratory Standards Institute (CLSI) recommendations.²² Non-susceptibility to ≥ 3 antibiotic classes was considered multidrug resistance (MDR). Serotyping was done by quellung

reaction.²³ The organisms were sub-cultured and stored at –70 °C at the Microbiology Laboratory of the Children's Hospital of Brasov and were further transported by air to the Pediatric Infectious Disease Unit Laboratory of the Soroka University Medical Center, Beer-Sheva, Israel, where all antimicrobial susceptibility testing and serotyping was carried out.

S. pneumoniae isolates were considered susceptible to penicillin if the minimal inhibitory concentration (MIC) values were ≤ 0.06 μ g/ml, intermediate if penicillin MIC values were between 0.125 μ g/ml and 1.0 μ g/ml, and resistant if MIC values were ≥ 2.0 μ g/ml. Ceftriaxone intermediate resistance was defined by MICs values between 0.5 and 1.0 μ g/ml, and high resistance by MICs values > 2.0 μ g/ml.

2.3. Statistical analysis

Data were recorded using Microsoft Access office software. The statistical analysis was performed using SPSS 17.0 software. Contingency table analysis for comparing rates between unmatched samples was performed using the Chi-square test or Fisher's exact test, as appropriate. The Student independent samples *t*-test was used to compare continuous variables. The percentages of serotype coverage were calculated and compared between PCV7 (serotypes 4, 6B, 9 V, 14, 18C, 19F, and 23F), PCV10 (PCV7 plus serotypes 1, 5, and 7F), and PCV13 (PCV10 plus additional serotypes 3, 6A, and 19A). All tests were considered significant if *p*-values were < 0.05 .

3. Results

During the study period, 212 consecutive infants and young children <5 years of age were enrolled. There were 120 (56.6%) males. The mean age (\pm standard deviation) was 18.0 ± 14.2 months. Ninety-nine (46.6%) episodes occurred in patients <12 months old and 136 (64.2%) episodes occurred in children <2 years old. One hundred and eleven samples (52.4%) were culture-positive. Children with culture-positive MEF were older than children with culture-negative MEF (20.6 ± 15.2 months vs. 15.4 ± 12.6 months, *p* = 0.008).

Tympanocentesis was recorded in 142 patients and spontaneous otorrhea in 70 patients. No differences were recorded in the proportions of spontaneous perforation between children with culture-positive and children with culture-negative MEF (38/111, 34.2% vs. 32/101, 31.7%, *p* = 0.7).

A total of 114 isolates (76 from tympanocentesis and 38 from spontaneous otorrhea specimens) were recovered. *S. pneumoniae* was the most common isolate (81 isolates, 78 episodes, 71.1% of all pathogens recovered and 70.3% of all culture-positive episodes), followed by NTHi (26, 23; 22.8% and 20.7%), *Streptococcus pyogenes* (5, 5; 4.4% and 4.5%) and *Moraxella catarrhalis* (2, 2; 1.8% and 1.8%) (Table 1). In three patients (1.4% of all episodes), both *S. pneumoniae* and NTHi were isolated. No differences were recorded in the percentages of *S. pneumoniae* recovered from

Table 1

Acute otitis media microbiology of 212 episodes (111 culture-positive) during 2009–2011

Pathogen	No. of episodes ^a
<i>Streptococcus pneumoniae</i>	78 (70.3)
<i>Haemophilus influenzae</i>	23 (20.7)
<i>Streptococcus pyogenes</i>	5 (4.5)
<i>Moraxella catarrhalis</i>	2 (1.8)
<i>S. pneumoniae</i> + <i>H. influenzae</i>	3 (2.7)
Culture-positive	111
Culture-negative	101
Total	212

^a The percentage of all culture-positive episodes is given in parenthesis.

tympanocentesis compared with those isolated from spontaneous otorrhea (55/76, 72.4% vs. 26/38, 68.4%, $p = 0.7$). No differences were recorded in the mean age at diagnosis of patients with *S. pneumoniae* AOM compared with patients with AOM caused by other etiologic agents (18.16 ± 7.08 vs. 24.59 ± 18.37 months, $p = 0.1$).

Reliable information on prior antibiotic treatment (during the 24 h preceding AOM diagnosis) was available for 68/111 (60.2%) culture-positive patients; 29/68 (42.6%) culture-positive patients received previous antibiotic treatment compared with 34/101 (33.7%) culture-negative patients ($p = 0.24$). The representation of *S. pneumoniae* was higher in patients previously untreated with antibiotics compared with patients treated with antibiotics (28/39, 71.7% vs. 14/29, 48.3%, $p = 0.03$).

Antibiotic susceptibility testing and serotyping were performed for 48/81 (59.3%) *S. pneumoniae* isolates. Forty-five (93.8%) *S. pneumoniae* isolates were non-susceptible to penicillin; 24 (53.3%) had MIC values ≥ 2.0 $\mu\text{g/ml}$. Six isolates had a penicillin MIC value of 16.0 $\mu\text{g/ml}$, one had a MIC of 8.0 $\mu\text{g/ml}$, and three had a MIC of 4.0 $\mu\text{g/ml}$. Thirty-seven (77.1%) isolates had ceftriaxone MIC values ≥ 0.5 $\mu\text{g/ml}$ (32.0 $\mu\text{g/ml}$ for five isolates, 4.0 $\mu\text{g/ml}$ for nine isolates, 2.0 $\mu\text{g/ml}$ for one isolate, and 1.0 $\mu\text{g/ml}$ for one isolate). The non-susceptibility rates to TMP–SMX, erythromycin, and clindamycin were 36/48 (75%), 28/48 (58.3%), and 17/48 (35.4%), respectively. All isolates were susceptible to chloramphenicol. Resistance to ≥ 1 antibiotic class was found in 44 (91.7%) and MDR in 33 (68.7%) *S. pneumoniae* isolates. No differences were recorded in the resistance patterns to antibiotics of *S. pneumoniae* recovered from tympanocentesis versus the isolates recovered from culture of spontaneous otorrhea.

The most common *S. pneumoniae* serotypes were: 19F (14, 29.2%), 6B (8, 16.7%), 23F (8, 16.7%), and 14 (6, 12.5%) (Table 2). Serotype 19A was found in three (6.2%) patients and 6A in two (4.1%) patients. The non-PCV13 serotypes represented six (12.6%) of all serotypes, and four of them were non-susceptible to penicillin. Six (75%) of the eight serotype 23F isolates had a penicillin MIC of 16.0 $\mu\text{g/ml}$ and a ceftriaxone MIC value of 32.0 $\mu\text{g/ml}$; 3/14 (21.4%) serotype 19F isolates had a penicillin MIC value of 4.0 $\mu\text{g/ml}$ and one had a penicillin MIC value of 8.0 $\mu\text{g/ml}$. The MDR isolates included, in descending order, serotypes 19F, 6B, 23F, 19A, and 6A. Of the 14 serotype 19F isolates, 13 (92.9%) were non-susceptible to penicillin, erythromycin, and TMP–SMX, and 9/13 (69.1%) also to clindamycin. All eight serotype 6B isolates were non-susceptible to penicillin, erythromycin, TMP–SMX, and clindamycin. All eight serotype 23F isolates were non-susceptible to penicillin, erythromycin, and TMP–SMX, and 5/8 (62.5%) also to clindamycin. All three serotype 19A isolates were non-susceptible to penicillin, erythromycin,

and TMP–SMX, and 2/3 (66.7%) also to clindamycin. One of the two serotype 6A isolates was non-susceptible to penicillin, erythromycin, TMP–SMX, and clindamycin.

Of the 48 tested *S. pneumoniae* serotype isolates, 36 (75.0%) are included in PCV7, 37 (77.0%) in PCV10, and 42 (87.5%) in PCV13.

4. Discussion

The present study aimed to provide baseline data on the contribution of *S. pneumoniae* to the etiology of AOM in Romania. We also analyzed the resistance patterns to antibiotics and the serotype distribution of this pathogen before the introduction of universal PCV immunization in Romania.

Our main findings were: (1) infants and young children with culture-positive AOM were older compared with those with culture-negative AOM; (2) *S. pneumoniae* was the dominant etiologic agent, being isolated in 74.3% of the culture-positive patients; (3) most of the isolates were resistant to penicillin, erythromycin, and TMP–SMX, and MDR was very common; (4) the current PCVs could potentially prevent most of the pneumococcal AOM in the region. The present study may provide a basis for follow-up and monitoring of AOM etiology and resistance patterns after the introduction of PCV into the national program.

In the Brasov area, we recently reported that of 205 pneumococcal nasopharyngeal isolates obtained from infants and children <5 years of age (attending daycare centers, immunization clinics, or visiting the pediatric emergency room of the hospital for different acute illnesses, as well as healthy patients admitted to the surgery department for elective procedures), 83% were non-susceptible to penicillin and 18% were non-susceptible to ceftriaxone, of which 40.5% and 16% were highly-resistant to penicillin and ceftriaxone, respectively.²¹ The non-susceptibility rates to erythromycin, TMP–SMX, tetracycline, and clindamycin were also high (>50% for each antibiotic) and MDR was found in 67% of isolates. The most common pneumococcal serotypes isolated were 23F, 6B, 19F, 14, 6A, and 19A, and the potential coverage by PCV7, PCV10, and PCV13 was 66%, 74%, and 80%, respectively. This is consistent with the AOM findings in the current study.

The major limitation of this study derives from the small number of pneumococcal isolates evaluable for antibiotic susceptibility testing and serotyping. On the other hand, the data presented here from MEF cultures performed in AOM patients diagnosed and treated at the Brasov Children's Hospital are additional to previously published information on the pneumococcal carriage in patients enrolled from daycare centers and immunization clinics in the city of Brasov and also from the emergency room and surgery department of the hospital (which is the only referral pediatric hospital in the whole area and is the only site where MEF and nasopharyngeal cultures are performed).²¹ Therefore, we are convinced that these two studies from Brasov provide an up-to-date and reliable picture of the local pneumococcal burden in infants and young children and the antibiotic susceptibility, serotype distribution, and potential serotype coverage by PCVs in the city of Brasov and the surrounding areas. At the present time, additional limited data from nasopharyngeal and MEF pneumococcal isolates obtained from infants and young children are available from the northeastern (Iasi) and southern (Bucharest) areas of the country and provide a similar picture in terms of colonization burden, extremely high antibiotic resistance rates, serotype distribution, and potential PCV coverage.^{17–21,24}

The presented data, together with the additional data from northeastern Romania, raise major concerns regarding the unskilled use of antibiotics in this country, leading to high resistance. Furthermore, the high rate of resistance is of concern in

Table 2
Streptococcus pneumoniae serotype distribution (in decreasing frequency) for 48 acute otitis media episodes

Serotype	No. episodes (%)	MDR
19F	14 (29.2)	13 (92.9%)
23F	8 (16.7)	8 (100%)
6B	8 (16.7)	8 (100%)
14	6 (12.5)	-
19A	3 (6.2)	3 (100%)
6A	2 (4.1)	1 (50%)
22F	2 (4.1)	-
9V	1 (2.1)	-
34	1 (2.1)	-
9A	1 (2.1)	-
7F	1 (2.1)	-
Omni-negative	1 (2.1)	-
Total	48	33 (68.7%)

MDR, multiple drug resistance.

terms of the efficacy of current antimicrobial agents in the treatment of AOM.

Faced with the alarming resistance data presented in this study, an intervention program including a major reduction in antibiotic use, combined with introduction of routine vaccination with PCVs, is much needed in Romania. In France, Cohen et al.²⁵ clearly showed that the implementation of a national program of reduction of inappropriate antibiotic use markedly contributed to the efficacy of PCV7 in reducing the carriage of penicillin-non-susceptible pneumococci in children with AOM. In a study evaluating the association between antibiotic use in the community and the increase in antibiotic-resistant *S. pneumoniae* carriage in Bedouin children aged <5 years in southern Israel from 1998 to 2005, Greenberg et al.²⁶ examined all the antibiotic prescriptions provided from two community primary pediatric clinics and reported a decrease by 19% in the total annual prescription rates, mainly as a result of a reduction in amoxicillin–clavulanate prescriptions. Oral cephalosporins, erythromycin, and penicillin prescription rates decreased significantly as well, but azithromycin prescription rates increased significantly during the study period. In parallel, the authors analyzed the *S. pneumoniae* nasopharyngeal carriage in healthy children <5 years old from the same communities and were able to demonstrate an increase in the proportion of nasopharyngeal *S. pneumoniae* with penicillin MICs ≥ 1.0 $\mu\text{g/ml}$ from 8% to 21% and significant increases in resistance to clindamycin, erythromycin, and tetracycline and also in multidrug resistance. The authors suggested an association between the increased carriage of MDR *S. pneumoniae* and the increased azithromycin consumption, and cautioned that a reduction in the total antibiotic use may not be sufficient as long as antibiotics with a high potential for the promotion of multidrug resistance, like azithromycin, continue to be used widely.²⁶

The introduction of the 7-valent conjugate PCV (PCV7) had a major role in the reduction of invasive and mucosal disease rates caused by *S. pneumoniae* and of the antimicrobial resistance of the isolated organisms, and also, although less impressive, in the reduction of nasopharyngeal colonization and AOM cases caused by this pathogen.^{27–32} In our study, the good coverage of overall pneumococcal serotypes and also of MDR pneumococcal isolates by all PCVs, and in particular by PCV13 (87.5% and 100%, respectively), are important and encouraging findings.

PCV7 was registered in Romania in September 2007, but is not yet included in the routine immunization program for Romanian infants and children. Initiation of a national immunization program is urgently needed in order to achieve a reduction in pneumococcal disease in general and of antibiotic-resistant and MDR pneumococcal AOM in particular.

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